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(54) Title: OSTEOGENIC PEPTIDES

(57) Abstract

Disclosed are 1) the cDNA and amino acid sequences for novel polypeptide chains useful as subunits of dimeric osteogenic proteins, 2) osteogenic devices comprising these proteins in association with an appropriate carrier matrix, 3) methods of producing the polypeptide chains using recombinant DNA technology, and 4) methods of using the osteogenic devices to mimic the natural course of endochondral bone formation in mammals.

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Osteogenic peptides

Background of the Invention

This invention relates to novel polypeptide chains and to osteogenic proteins comprising these polypeptide chains which are capable of inducing osteogenesis in mammals; to genes encoding the polypeptide chains; to methods for their production using recombinant DNA techniques, and to bone and cartilage repair procedures using the osteogenic proteins.

Mammalian bone tissue is known to contain one or more proteinaceous materials, presumably active during growth and natural bone healing, which can induce a developmental cascade of cellular events resulting in endochondral bone formation. This active factor (or factors) has variously been referred to in the literature as bone morphogenetic or morphogenic protein, bone inductive protein, osteogenic protein, osteogenin, or osteoinductive protein.

The developmental cascade of bone differentiation consists of recruitment of mesenchymal cells, proliferation of progenitor cells, calcification of cartilage, vascular invasion, bone formation, remodeling, and finally marrow differentiation (Reddi (1981) Collagen Rel. Res. 1:209-226).

Though the precise mechanisms underlying these phenotypic transformations are unclear, it has been shown that the natural endochondral bone differentiation activity of bone matrix can be dissociatively extracted and reconstituted with

inactive residual collagenous matrix to restore full bone induction activity (Sampath and Reddi, (1981) Proc. Natl. Acad. Sci. USA 78:7599-7603). This provides an experimental method for assaying protein extracts for their ability to induce endochondral bone in vivo. Several species of mammals produce closely related protein as demonstrated by cross species implant experiments (Sampath and Reddi (1983) Proc. Natl. Acad. Sci. USA 80:6591-6595).

The potential utility of these proteins has been recognized widely. It is contemplated that the availability of the protein would revolutionize orthopedic medicine, certain types of plastic surgery, and various periodontal and craniofacial reconstructive procedures.

The observed properties of these protein fractions have induced an intense research effort in various laboratories directed to isolating and identifying the pure factor or factors responsible for osteogenic activity. The current state of the art of purification of osteogenic protein from mammalian bone is disclosed by Sampath et al. ((1987) Proc. Natl. Acad. Sci. USA 84: 7109-7113). Urist et al. (1984) Proc. Soc. Exp. Biol. Med. 173: 194-199 disclose a human osteogenic protein fraction which was extracted from demineralized cortical bone by means of a calcium chloride-urea inorganic-organic solvent mixture, and retrieved by differential precipitation in guanidine-hydrochloride and preparative gel electrophoresis. The authors report that the protein fraction has an amino acid composition of an acidic polypeptide and a molecular weight in a range of 17-18 kD.

Urist et al. (1984) Proc. Natl. Acad. Sci. USA 81: 371-375 disclose a bovine bone morphogenetic protein extract having the properties of an acidic polypeptide and a molecular weight of approximately 18 kD. The authors reported that the protein was present in a fraction separated by hydroxyapatite chromatography, and that it induced bone formation in mouse hindquarter muscle and bone regeneration in trephine defects in rat and dog skulls. Their method of obtaining the extract from bone results in ill-defined and impure preparations.

European Patent Application Serial No. 148,155, published October 7, 1985, purports to disclose osteogenic proteins derived from bovine, porcine, and human origin. One of the proteins, designated by the inventors as a P3 protein having a molecular weight of 22-24 kD, is said to have been purified to an essentially homogeneous state. This material is reported to induce bone formation when implanted into animals.

International Application No. PCT/087/01537, published January 14, 1988, discloses an impure fraction from bovine bone which has bone induction qualities. The named applicants also disclose putative "bone inductive factors" produced by recombinant DNA techniques. Four DNA sequences were retrieved from human or bovine genomic or cDNA libraries and expressed in recombinant host cells. While the applicants stated that the expressed proteins may be bone morphogenic proteins, bone induction was not demonstrated, suggesting that the recombinant proteins are not osteogenic. The same group reported subsequently (Science, 242:1528, Dec, 1988) that three of the four

factors induce cartilage formation, and postulate that bone formation activity "is due to a mixture of regulatory molecules" and that "bone formation is most likely controlled ... by the interaction of these molecules." Again, no bone induction was attributed to the products of expression of the cDNAs. See also Urist et al., EP0,212,474 entitled Bone Morphogenic Agents.

Wang et al. (1988) Proc. Nat. Acad. Sci. USA 85: 9484-9488 discloses the purification of a bovine bone morphogenetic protein from guanidine extracts of demineralized bone having cartilage and bone formation activity as a basic protein corresponding to a molecular weight of 30 kD determined from gel elution. Purification of the protein yielded proteins of 30, 18 and 16 kD which, upon separation, were inactive. In view of this result, the authors acknowledged that the exact identity of the active material had not been determined.

Wang et al. (1990) Proc. Nat. Acad. Sci. USA 87: 2220-2227 describes the expression and partial purification of one of the cDNA sequences described in PCT 87/01537. Consistent cartilage and/or bone formation with their protein requires a minimum of 600 ng of 50% pure material.

International Application No. PCT/89/04458 published April 19, 1990 (Int. Pub. No. WO90/003733), describes the purification and analysis of a family of osteogenic factors called "P3 OF 31-34". The protein family contains at least four proteins, which are characterized by peptide fragment sequences. The impure mixture P3 OF 31-34 is assayed for osteogenic

activity. The activity of the individual proteins is neither assessed nor discussed.

It is an object of this invention to provide novel polypeptide chains useful as subunits of dimeric osteogenic proteins capable of endochondral bone formation in allogenic and xenogenic implants in mammals, including humans. Another object is to provide genes encoding these polypeptide chains and methods for the production of osteogenic proteins comprising these polypeptide chains using recombinant DNA techniques, as well as to provide antibodies capable of binding specifically to these proteins.

These and other objects and features of the invention will be apparent from the description, drawings, and claims which follow.

Summary of the Invention

This invention provides novel polypeptide chains useful as either one or both subunits of dimeric osteogenic proteins which, when implanted in a mammalian body in association with a matrix, can induce at the locus of the implant the full developmental cascade of endochondral bone formation and bone marrow differentiation.

A key to these developments was the elucidation of amino acid sequence and structure data of native bovine osteogenic protein. A protocol was developed which results in retrieval of active, substantially pure osteogenic protein from bovine bone having a half-maximum bone forming activity of about 0.8 to 1.0 ng per mg of implant. The availability of the material enabled the inventors to elucidate key structural details of the protein necessary to achieve bone formation. Knowledge of the protein's amino acid sequence and other structural features enabled the identification and cloning of native genes in the human genome.

Consensus DNA sequences based on partial sequence data and observed homologies with regulatory proteins disclosed in the literature were used as probes for extracting genes encoding osteogenic protein from human genomic and cDNA libraries. One of the consensus sequences was used to isolate a previously unidentified gene which, when expressed, encoded a protein comprising a region capable of inducing endochondral bone formation when properly modified, incorporated in a suitable matrix, and implanted as disclosed herein. The gene, called "hOP1" or "OP-1" (human OP-1), is

described in greater detail in copending U.S. 422,699, the disclosure of which is herein incorporated by reference.

In its native form, hOP1 expression yields an immature translation product ("hOP1-PP", where "PP" refers to "prepro form") of about 400 amino acids that subsequently is processed to yield a mature sequence of 139 amino acids ("OP1-18"). The active region (functional domain) of the protein comprises the C-terminal 97 amino acids of the hOP1 sequence ("OPS"). A long active sequence is OP7 (comprising the C-terminal 102 amino acids).

Further probing of mammalian cDNA libraries (human and mouse) with sequences specific to hOP1 also has identified novel OP1-like sequences herein referred to as "OP2" ("hOP2" or "mOP2"). The OP2 proteins share significant amino acid sequence homology, approximately 74%, with the active region of the OP1 proteins (e.g., OP7), and less homology with the intact mature form (e.g., OP1-18, 58% amino acid homology).

The amino acid sequence of the osteogenic proteins disclosed herein also share significant homology with various of the regulatory proteins on which the consensus probe was modeled. In particular, the proteins share significant homology in their C-terminal sequences, which comprise the active region of the osteogenic proteins. (Compare, for example, OP7 with DPP from Drosophila and Vgl from Xenopus. See, for example, U.S. Pat. No. 5,011,691). In addition, these proteins share a conserved six or seven cysteine skeleton in this region (e.g., the linear arrangement of these C-terminal cysteine residues is conserved in

the different proteins.) See, for example, OP7, whose sequence defines the seven cysteine skeleton, or OPS, whose sequence defines the six cysteine skeleton. The OP2 proteins also contain an additional cysteine residue within this region.

Thus, in one preferred aspect, the invention comprises osteogenic proteins comprising a polypeptide chain comprising an amino acid sequence described by Seq. ID No. 3 or 5, including allelic and species variants thereof, and naturally-occurring or biosynthetic mutants, such that a dimeric protein comprising this polypeptide chain has a conformation capable of inducing endochondral bone formation when implanted in a mammal in association with a suitable matrix. Useful proteins include the full-length protein, mature proteins and truncated proteins comprising the functional domain described by the C-terminal.

In addition, the invention is not limited to thse specific constructs. Thus, the osteogenic proteins of this invention comprising any of these polypeptide chains may include forms having varying glycosylation patterns, varying N-termini, a family of related proteins having regions of amino acid sequence homology which may be naturally occurring or biosynthetically derived, and active truncated or mutated forms of the native amino acid sequence, produced by expression of recombinant DNA in procaryotic or eucaryotic host cells. Active squances useful as osteogenic proteins of this invention are envisioned to include proteins capable of inducing endochondral bone formation when implanted in a mammal in association wiht a matrix and having at lest a 70% sequence homology, preferably at

least 80%, with the amino acid sequence of OPS. This includes longer forms of a given protein, as well as allelic variants and muteins, including addition and deletion mutants, such as those which may alter the conserved C-terminal cysteine skeleton, provided that the alteration still allows the protein to form a dimeric species having a conformation capable of inducing bone formation in a mammal when implanted in the mammal in association with a matrix.

The novel polypeptide chains and the osteogenic \roteins they comprise can be expressed from intact or truncated cDNA or from synthetic DNAs in procaryotic or eucaryotic host cells, and then purified, cleaved, refolded, dimerized, and implanted in experimental animals. Currently preferred host cells include E.coli or mammalian cells, such as CHO, COS or BSC cells. The osteogenic protein of the invention may include forms having varying glycosylation patterns, varying N-termini, a family of related proteins having regions of amino acid sequence homology, and active truncated or mutated forms of native or biosynthetic proteins, produced by expression of recombinant DNA in host cells.

Thus, in view of this disclosure, skilled genetic engineers can isolate genes from cDNA or genomic libraries of various different species which encode appropriate amino acid sequences, or construct DNAs from oligonucleotides, and then can express them in various types of host cells, including both procaryotes and eucaryotes, to produce large quantities of active proteins capable of inducing bone formation in mammals including humans. In view of this disclosure, those skilled in the art, using standard immunology

techniques also may create antibodies capable of binding specifically to the osteogenic proteins disclosed herein, including fragments thereof.

The osteogenic proteins are useful in clinical applications in conjunction with a suitable delivery or support system (matrix). The matrix is made up of particles of porous materials. The pores must be of a dimension to permit progenitor cell migration and subsequent differentiation and proliferation. The particle size should be within the range of 70 - 850 mm, preferably 150mm - 420mm. It may be fabricated by close packing particulate material into a shape spanning the bone defect, or by otherwise structuring as desired a material that is biocompatible (non-inflammatory) and, biodegradable in vivo to serve as a "temporary scaffold" and substratum for recruitment of migratory progenitor cells, and as a base for their subsequent anchoring and proliferation. Currently preferred carriers include particulate, demineralized, guanidine extracted, species-specific (allogenic) bone, and specially treated particulate, protein extracted, demineralized, xenogenic bone. Optionally, such xenogenic bone powder matrices also may be treated with proteases such as trypsin and/or fibril modifying agents to increase the intraparticle intrusion volume and surface area. Useful agents include solvents such as dichloromethane, trichloroacetic acid, acetonitrile and acids such as trifluoroacetic acid and hydrogen fluoride. Alternatively, the matrix may be treated with a hot aqueous medium having a temperature within the range of about 37°C to 75°C, including a heated acidic aqueous medium. Other potentially useful matrix materials comprise collagen, homopolymers and copolymers of glycolic acid and lactic acid,

hydroxyapatite, tricalcium phosphate and other calcium phosphates.

The osteogenic proteins and implantable osteogenic devices enabled and disclosed herein will permit the physician to obtain optimal predictable bone formation to correct, for example, acquired and congenital craniofacial and other skeletal or dental anomalies (Glowacki et al. (1981) Lancet 1:959-963). The devices may be used to induce local endochondral bone formation in non-union fractures as demonstrated in animal tests, and in other clinical applications including dental and periodontal applications where bone formation is required. Another potential clinical application is in cartilage repair, for example, in the treatment of osteoarthritis.

Brief Description of the Drawing

The foregoing and other objects of this invention, the various features thereof, as well as the invention itself, may be more fully understood from the following description, when read together with the accompanying drawings, in which:

FIGURE 1 compares the amino acid sequences of the mature mOP-2 and hOP-2 polypeptide chains: hOP2-A and mOP2-A; and

FIGURE 2 compares the amino acid sequences of the mature OP1 and OP2 polypeptide chains: OP1-18, mOP1-S, hOP2-A and mOP2-A.

Description

Purification protocols first were developed which enabled isolation of the osteogenic protein present in crude protein extracts from mammalian bone. (See PCT WO 89/09787, published 19-OCT-89, and U.S. Serial No. 179,406 filed April 8, 1988, now U.S. Patent No. 4,968,950). The development of the procedure, coupled with the availability of fresh calf bone, enabled isolation of substantially pure bovine osteogenic protein (bOP). bOP was characterized significantly; its ability to induce cartilage and ultimately endochondral bone growth in cat, rabbit, and rat were demonstrated and studied; it was shown to be able to induce the full developmental cascade of bone formation previously ascribed to unknown protein or proteins in heterogeneous bone extracts. This dose dependent and highly specific activity was present whether or not the protein was glycosylated (see Sampath et al., (1990) J. Biol. Chem. 265: 13198-13205). Sequence data obtained from the bovine materials suggested probe designs which were used to isolate human genes. The OP human counterpart proteins have now been expressed and extensively characterized.

These discoveries enabled preparation of DNAs encoding totally novel, non-native protein constructs which individually as homodimers and combined with other species as heterodimers are capable of producing true endochondral bone (see PCT WO 09788, published 19-OCT-89, and US Serial No. 315,342, filed 23-FEB-89, now U.S. Patent No. 5,011,691). They also permitted expression of the natural material, truncated forms, muteins, analogs, fusion proteins, and various other variants and constructs, from cDNAs and genomic DNAs retrieved from natural sources or from synthetic DNA produced using the techniques disclosed herein and

using automated, commercially available equipment. The DNAs may be expressed using well established molecular biology and recombinant DNA techniques in procaryotic or eucaryotic host cells, and may be oxidized and refolded in vitro if necessary, to produce biologically active protein.

One of the DNA sequences isolated from human genomic and cDNA libraries encoded a previously unidentified gene, referred to herein as OP1. The protein encoded by the isolated DNA was identified originally by amino acid homology with proteins in the TGF-β family. Consensus splice signals were found where amino acid homologies ended, designating exon-intron boundaries. Three exons were combined to obtain a functional TGF-β-like domain containing seven cysteines. (See, for example, U.S. Patent No. 5,011,691, or Ozkaynak, E. et al., (1990) EMBO. 9: 2085-2093).

The full-length cDNA sequence for hOP1, and its encoded "prepro" form "hOP1-PP," which includes an N-terminal signal peptide sequence, are disclosed in Seq. ID No. 1 (residues 1-431). The mature form of the hOP1 protein expressed in mammalian cells, "OP1-18", is described by amino acid residues 293-431 of Seq. ID No. 1. The full length form of hOP1, as well as various truncated forms of the gene, and fused genes, have been expressed in *E. coli* and numerous mammalian cells (see, for example, published PCT application WO 91/05802, published 2-MAY-91) and all have been shown to have osteogenic activity when implanted in a mammal in association with a suitable matrix.

Given the foregoing amino acid and DNA sequence information, various nucleic acids (RNAs and DNAs) can be constructed which encode at least the active region of the hOP1 protein (e.g., OPS or OP7) and various analogs thereof (including allelic and species variants and those containing genetically engineered mutations), as well as fusion proteins, truncated forms of the mature proteins, and similar constructs. Moreover, DNA hybridization probes can be constructed from fragments of the hOP1 DNA or designed <u>de novo</u> based on the hOP1 DNA or amino acid sequence. These probes then can be used to screen different genomic and cDNA libraries to identify additional osteogenic proteins.

The DNAs can be produced by those skilled in the art using well known DNA manipulation techniques involving genomic and cDNA isolation, construction of synthetic DNA from synthesized oligonucleotides, and cassette mutagenesis techniques. 15-100mer oligonucleotides may be synthesized on a Biosearch DNA Model 8600 Synthesizer, and purified by polyacrylamide gel electrophoresis (PAGE) in Tris-Borate-EDTA buffer. The DNA then may be electroeluted from the gel. Overlapping oligomers may be phosphorylated by T4 polynucleotide kinase and ligated into larger blocks which may also be purified by PAGE.

DNAs used as hybridization probes may be labelled (e.g., as with a radioisotope, by nick-translation) and used to identify clones in a given library containing DNA to which the probe hybridizes, following techniques well known in the art. The libraries may be obtained commercially or they may constructed de novo using conventional molecular biology techniques. Further information on DNA library construction and

hybridization techniques can be found in numerous texts known to those skilled in the art. See, for example, F.M. Ausubel., ed., Current Protocols in Molecular Biology-Vol. 1, (1989). In particular, see unit 5, "Construction of Recombinant DNA Libraries" and Unit 6, "Screening of Recombinant Libraries."

The DNA from appropriately identified clones then can be isolated, subcloned (preferably into an expression vector), and sequenced. Plasmids containing sequences of interest then can be transfected into an appropriate host cell for protein expression and further characterization. The host may be a procaryotic or eucaryotic cell since the former's inability to glycosylate protein will not destroy the protein's osteogenic activity. Useful host cells include *E. coli*, *Saccharomyces*, the insect/baculovirus cell system, myeloma cells, and various mammalian cells. The vector additionally may encode various sequences to promote correct expression of the recombinant protein, including transcription promoter and termination sequences, enhancer sequences, preferred ribosome binding site sequences, preferred mRNA leader sequences, preferred signal sequences for protein secretion, and the like. The DNA sequence encoding the gene of interest also may be manipulated to remove potentially inhibiting sequences or to minimize unwanted secondary structure formation. The recombinant osteogenic protein also may be expressed as a fusion protein. After being translated, the protein may be purified from the cells themselves or recovered from the culture medium. All biologically active protein forms comprise dimeric species joined by disulfide bonds or otherwise associated, produced by oxidizing and refolding one or more of the various

recombinant polypeptide chains within an appropriate eucaryotic cell or <u>in vitro</u> after expression of individual subunits. A detailed description of osteogenic protein expressed from recombinant DNA in <u>E. coli</u> is disclosed in U.S. Serial No. 660,162, filed 27-FEB-91, the disclosure of which incorporated by reference herein. A detailed description of osteogenic protein expressed from recombinant DNA in numerous different mammalian cells is disclosed in PCT WO91/05802, also incorporated herein by reference.

Finally, in view of the disclosure made herein, and using standard methodologies known in the art, persosn skilled inthe art can raise polyclonal and monoclonal antibodies against all or part of a polypeptide chain disclosed herein, such that the antibodies are capable of binding specifically to an epitope on the polypeptide chain. Useful protocols can be found in, for example, <u>Molecular Cloing-A Laboratory Manual</u> (Sambrook et al. eds., Cold Spring Harbor Press 2nd ed. 1989). See Book 3, Section 18.

<u>Exemplification</u>

In an effort to identify additional DNA sequences encoding osteogenic proteins, a hybridization probe specific to the C-terminus of the DNA of mature OP-1 was prepared using a StuI-EcoR1 digest fragment of OP-1 (base pairs 1034-1354 in Sequence ID No. 1), and labelled with ^{32}P by nick translation, as described in the art. As disclosed supra, the OP1 C-terminus encodes a key functional domain, e.g., the "active region" for osteogenic activity. The C-terminus also is the region of the protein whose amino acid sequence shares specific amino acid sequence homology with

particular proteins in the TGF-β super-family of regulatory proteins, and which includes the conserved cysteine skeleton.

Approximately 7 x 10^5 phages of an oligo(dT) primed 17.5 days p.c. mouse embryo 5' stretch cDNA (gt10) library (Clonetech, Inc., Palo Alto, CA) was screened with the labelled probe. The screen was performed using the following stringent hybridization conditions: 40% formamide, 5 x SSPE, 5 x Denhart's solution, 0.1% SDS, at 37°C overnight, and washing in 0.1 x SSPE, 0.1% SDS at 50°C.

Five recombinant phages were purified over three rounds of screening. Phage DNA was prepared from all five phages, subjected to an EcoR1 digest, subcloned into the EcoR1 site of a common pUC-type plasmid modified to allow single strand sequencing, and sequenced using means well known in the art.

Two different DNAs were identified by this procedure. One DNA, referred to herein as mOP1, has substantial homology to the mature form of OP1 (about 98%), and is described in detail in copending USSN 600,024, filed 18-Oct-90. A second DNA, encoding the C-terminus of a related gene and referred to herein as mOP2, also was identified by this procedure. The N-terminus of the gene encoding mOP2 was identified subsequently by screening a second mouse cDNA library (Mouse PCC4 cDNA (ZAP) library, Stratagene, Inc., La Jolla, CA).

Mouse OP2 (mOP2) protein shares significant amino acid sequence homology with the amino acid sequence of the hOP1 active region, e.g., OPS or OP7, about 74%

homology, and less homology with the intact mature form, e.g., OP1-18, about 58% homology. The cDNA sequence, and the encoded amino acid sequence, for the full length mOP-2 protein is depicted in Sequence ID No. 3. The full-length form of the protein is referred to as the prepro form of mOP-2 ("mOP2-PP"), and includes a signal peptide sequence at its N-terminus. The amino acid sequence Leu-Ala-Leu-Cys-Ala-Leu (amino acid residues 13-18 of Sequence ID No. 3) is believed to constitute the cleavage site for the removal of the signal peptide sequence, leaving an intermediate form of the protein, the "pro" form, to be secreted from the expressing cell. The amino acid sequence Arg-Ala-Pro-Arg-Ala (amino acid residues 255-259 of Sequence ID No. 3) is believed to constitute the cleavage site that produces the mature form of the protein, herein referred to as "mOP2-A", and described by residues 259-397 of Seq. ID No. 3. Residues 301-397 of Seq. ID No. 3 correspond to the region defining the conserved six cysteine skeleton. Residues 296-397 of Seq. ID No. 3 correspond to the region defining the conserved seven cysteine skeleton.

Using a probe prepared from the pro region of mOP2 (an EcoR1-BamH1 digest fragment, bp 467-771 of Sequence ID No. 3), a human hippocampus library was screened (human hippocampus cDNA lambda (ZAP II library Stratagene, Inc., La Jolla, CA) following essentially the same procedure as for the mouse library screens. The procedure identified the N-terminus of a novel DNA encoding an amino acid sequence having substantial homology with mOP2. The C-terminus of the gene subsequently was identified by probing a human genomic library (in lambda phage EMBL-3, Clonetech, Inc., Palo Alto, CA) with a labelled fragment from the novel human

DNA in hand. The novel polypeptide chain encoded by this DNA is referred to herein as hOP2 protein, and shares almost complete amino acid identity (about 92% amino acid sequence homology) with mOP2-A (see Fig. 1 and infra).

The cDNA sequence, and the encoded amino acid sequence, for the prepro form of hOP2, "hOP2-PP", is described in Sequence ID No. 5. This full-length form of the protein also includes a signal peptide sequence at its N-terminus. The amino acid sequence Leu-Ala-Leu-Cys-Ala-Leu (amino acid residues 13-18 of Sequence ID No. 5) is believed to constitute the cleavage site for the removal of the signal peptide sequence, leaving an intermediate form of the protein, the "pro" form, to be secreted from the expressing cell. The amino acid sequence Arg-Thr-Pro-Arg-Ala (amino acid residues 257-261 of Sequence ID No. 5) is believed to constitute the cleavage site that produces what is believed to be the mature form of the protein, herein referred to as hOP2-A" and described by residues 261-399 of Seq. ID No. 5.

Additional mature species of hOP2 thought to be active include truncated sequences, "hOP2-P" (described by residues 264-399 of Seq. ID No. 5) and "hOP2-R" (described by residues 267-399 of Seq. ID No. 5), and a slightly longer sequence ("hOP2-S", described by residues 240-399 of Seq. ID No. 5). Residues 303-399 of Seq. ID No. 5 correspond to the region defining the conserved six cysteine skeleton. Residues 297-399 of Seq. ID No. 5 correspond to the region defining the conserved seven cystein skeleton.

It should be noted that the nucleic acid sequence encoding the N-terminus of the prepro form of both mOP2 and hOP2 is rich in guanidine and cytosine base pairs. As will be appreciated by those skilled in the art, sequencing such a "G-C rich" region can be problematic, due to stutter and/or band compression. Accordingly, the possibility of sequencing errors in this region can not be ruled out. However, the definitive amino acid sequence for these and other, similarly identified proteins can be determined readily by expressing the protein from recombinant DNA using, for example, any of the means disclosed herein, and sequencing the polypeptide chain by conventional peptide sequencing methods well known in the art.

Figure 1 compares the amino acid sequences of mature mOP2 and hOP2. Identity is indicated by three dots (...) in the mOP2 sequence. As is evident from the figure, the amino acid sequence homology between the mature forms of these two proteins is substantial (92% homology between the mature sequences, about 95% homology within the C-terminal active region (e.g., residues 38-139 or 42-139 of Fig. 1.)

Fig. 2 compares the amino acid sequences for the mature forms of all four species of OP1 and OP2 proteins. Here again, identity is indicated by three dots (...). Like the mOP2 protein, the hOP2 protein shares significant homology (about 74%) with the amino acid sequence defining the OP1 active region (OPS or OP7, residues 43-139 and 38-139, respectively, in Fig. 2), and less homology with OP1-18 (about 58% homology). Both OP2 proteins share the conserved seven cysteine skeleton seen in the OP1 proteins. In

addition, the OP2 proteins comprise an eighth cysteine residue within this region (see position 78 in FIG. 2).

A preferred generic amino acid sequence useful as a subunit of a dimeric osteogenic protein capable of inducing endochondral bone or cartilage formation when implanted in a mammal in association with a matrix, and which incorporates the maximum homology between the identified OP1 and OP2 proteins, can be described by the sequence referred to herein as "OPX", described below and in Seq. No.7.

```
Cys Xaa Xaa His Glu Leu Tyr Val Xaa Phe
 1               5                  10
Xaa Asp Leu Gly Trp Xaa Asp Trp Xaa Ile
                15                  20
Ala Pro Xaa Gly Tyr Xaa Ala Tyr Tyr Cys
                25                  30
Glu Gly Glu Cys Xaa Phe Pro Leu Xaa Ser
                35                  40
Xaa Met Asn Ala Thr Asn His Ala Ile Xaa
                45                  50
Gln Xaa Leu Val His Xaa Xaa Xaa Pro Xaa
                55                  60
Xaa Val Pro Lys Xaa Cys Cys Ala Pro Thr
                65                  70
Xaa Leu Xaa Ala Xaa Ser Val Leu Tyr Xaa
                75                  80
Asp Xaa Ser Xaa Asn Val Xaa Leu Xaa Lys
                85                  90
Xaa Arg Asn Met Val Val Xaa Ala Cys Gly
                95                 100
Cys His,
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and wherein Xaa at res. 2 = (Lys or Arg); Xaa at res. 3 = (Lys or Arg); Xaa at res. 9 = (Ser or Arg); Xaa at res. 11 = (Arg or Gln); Xaa at res. 16 = (Gln or Leu); Xaa at res. 19 = (Ile or Val); Xaa at res. 23 = (Glu or Gln); Xaa at res. 26 = (Ala or Ser); Xaa at res. 35 = (Ala or Ser); Xaa at res. 39 = (Asn or Asp); Xaa at res. 41 = (Tyr or Cys); Xaa at res. 50 = (Val or Leu); Xaa at res. 52 = (Ser or Thr); Xaa at res. 56 = (Phe or Leu); Xaa at res. 57 = (Ile or Met); Xaa at res. 58 = (Asn or Lys); Xaa at res. 60 = (Glu, Asp or Asn); Xaa at res. 61 = (Thr, Ala or Val); Xaa at res. 65 = (Pro or Ala); Xaa at res. 71 = (Gln or Lys); Xaa at res. 73 = (Asn or Ser); Xaa at res. 75 = (Ile or Thr); Xaa at res. 80 = (Phe or Tyr); Xaa at res. 82 = (Asp or Ser); Xaa at res. 84 = (Ser or Asn); Xaa at res. 87 = (Ile or Asp); Xaa at res. 89 = (Lys or Arg); Xaa at res. 91 = (Tyr, Ala or His); and Xaa at res. 97 = (Arg or Lys).

The high degree of homology exhibited between the various OP1 and OP2 proteins suggests that the novel osteogenic proteins identified herein will purify essentially as OP1 does, or with only minor modifications of the protocols disclosed for OP1. Similarly, the purified mOP1, mOP2, and hOP2 proteins are predicted to have an apparent molecular weight of about 18 kDa as reduced single subunits, and an apparent molecular weight of about 36 kDa as oxidized dimers, as determined by comparison with molecular weight standards on an SDS-polyacrylamide electrophoresis gel. Unglycosylated dimers (e.g., proteins produced by recombinant expression in _E. coli_) are predicted to have an apparent molecular weight of about 27 kDa. There appears to be one potential N

glycosylation site in the mature forms of the mOP2 and hOP2 proteins.

The identification of osteogenic proteins having an active region comprising eight cysteine residues also allows one to construct osteogenic polypeptide chains patterned after either of the following template amino acid sequences, or to identify additional osteogenic proteins having this sequence. The template sequences contemplated are "OPX-7C", comprising the conserved six cysteine skeleton plus the additional cysteine residue identified in the OP2 proteins, and "OPX-8C", comprising the conserved seven cysteine skeleton plus the additional cysteine residue identified in the OP2 proteins. The OPX-7C and OPX-8C sequences are described below and in Seq. ID Nos. 8 and 9, respectively. Each Xaa in these template sequences independently represents one of the 20 naturally-occurring L-isomer, α-amino acids, or a derivative thereof. Biosynthetic constructs patterned after this template readily are constructed using conventional DNA synthesis or peptide synthesis techniques well known in the art. Once constructed, osteogenic proteins comprising these polypeptide chains can be tested as disclosed herein.

"OPX-7C" (Sequence ID No. 8):

```
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
 1               5                  10
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
            15                  20
Xaa Xaa Cys Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa
        25                  30
Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
```

35 40
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
45 50 55
Xaa Xaa Xaa Xaa Xaa Cys Cys Xaa Xaa Xaa Xaa
60 65
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
70 75
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
80 85
Xaa Xaa Xaa Xaa Xaa Cys Xaa Cys Xaa
90 95

"OPX-8C" (Sequence ID No. 9 comprising additional five residues at the N-terminus, including a conserved cysteine residue):

Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5 10
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
15 20
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa
25 30
Cys Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa
35 40 45
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
50 55
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
60 65
Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
70 75
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
80 85
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
90 95
Xaa Cys Xaa

100

MATRIX PREPARATION

A. General Consideration of Matrix Properties

The currently preferred carrier material is a xenogenic bone-derived particulate matrix treated as disclosed herein. This carrier may be replaced by either a biodegradable-synthetic or synthetic-inorganic matrix (e.g., hydroxylapatite (HAP), collagen, tricalcium phosphate or polylactic acid, polyglycolic acid and various copolymers thereof.)

Studies have shown that surface charge, particle size, the presence of mineral, and the methodology for combining matrix and osteogenic protein all play a role in achieving successful bone induction. Perturbation of the charge by chemical modification abolishes the inductive response. Particle size influences the quantitative response of new bone; particles between 75 μm and 420 μm elicit the maximum response. Contamination of the matrix with bone mineral will inhibit bone formation. Most importantly, the procedures used to formulate OP onto the matrix are extremely sensitive to the physical and chemical state of both the osteogenic protein and the matrix.

The sequential cellular reactions in the interface of the bone matrix/osteogenic protein implants are complex. The multistep cascade includes: binding of fibrin and fibronectin to implated matrix, chemotaxis of cells, proliferation of fibroblasts, differentiation into chondroblasts, cartilage

formation, vascular invasion, bone formation, remodeling, and bone marrow differentiation.

A successful carrier for osteogenic protein must perform several important functions. It must bind osteogenic protein and act as a slow release delivery system, accommodate each step of the cellular response during bone development, and protect the osteogenic protein from nonspecific proteolysis. In addition, selected materials must be biocompatible in vivo and preferably biodegradable; the carrier must act as a temporary scaffold until replaced completely by new bone. Polylactic acid (PLA), polyglycolic acid (PGA), and various combinations have different dissolution rates in vivo. In bones, the dissolution rates can vary according to whether the implant is placed in cortical or trabecular bone.

Matrix geometry, particle size, the presence of surface charge, and the degree of both intra-and-inter-particle porosity are all important to successful matrix performance. It is preferred to shape the matrix to the desired form of the new bone and to have dimensions which span non-union defects. Rat studies show that the new bone is formed essentially having the dimensions of the device implanted.

The matrix may comprise a shape-retaining solid made of loosely adhered particulate material, e.g., with collagen. It may also comprise a molded, porous solid, or simply an aggregation of close-packed particles held in place by surrounding tissue. Masticated muscle or other tissue may also be used. Large allogenic bone implants can act as a carrier for the matrix if their marrow cavities are cleaned and

packed with particle and the dispersed osteogenic protein.

The preferred matrix material, prepared from xenogenic bone and treated as disclosed herein, produces an implantable material useful in a variety of clinical settings. In addition to its use as a matrix for bone formation in various orthopedic, periodontal, and reconstructive procedures, the matrix also may be used as a sustained release carrier, or as a collagenous coating for implants. The matrix may be shaped as desired in anticipation of surgery or shaped by the physician or technician during surgery. Thus, the material may be used for topical, subcutaneous, intraperitoneal, or intramuscular implants; it may be shaped to span a nonunion fracture or to fill a bone defect. In bone formation or conduction procedures, the material is slowly absorbed by the body and is replaced by bone in the shape of or very nearly the shape of the implant.

Various growth factors, hormones, enzymes, therapeutic compositions, antibiotics, and other body treating agents also may be absorbed onto the carrier material and will be released over time when implanted as the matrix material is slowly absorbed. Thus, various known growth factors such as EGF, PDGF, IGF, FGF, TGF-α, and TGF-ß may be released <u>in vivo</u>. The material can be used to release chemotherapeutic agents, insulin, enzymes, or enzyme inhibitors.

B. Bone-Derived Matrices

1. Preparation of Demineralized Bone

Demineralized bone matrix, preferably bovine bone matrix, is prepared by previously published procedures (Sampath and Reddi (1983) Proc. Natl. Acad. Sci. USA 80:6591-6595). Bovine diaphyseal bones (age 1-10 days) are obtained from a local slaughterhouse and used fresh. The bones are stripped of muscle and fat, cleaned of periosteum, demarrowed by pressure with cold water, dipped in cold absolute ethanol, and stored at -20°C. They are then dried and fragmented by crushing and pulverized in a large mill. Care is taken to prevent heating by using liquid nitrogen. The pulverized bone is milled to a particle size in the range of 70-850 μm, preferably 150-420 μm, and is defatted by two washes of approximately two hours duration with three volumes of chloroform and methanol (3:1). The particulate bone is then washed with one volume of absolute ethanol and dried over one volume of anhydrous ether yielding defatted bone powder. The defatted bone powder is then demineralized by four successive treatments with 10 volumes of 0.5 N HCl at 4°C for 40 min. Finally, neutralizing washes are done on the demineralized bone powder with a large volume of water.

2. Guanidine Extraction

Demineralized bone matrix thus prepared is extracted with 5 volumes of 4 M guanidine-HCl, 50mM Tris-HCl, pH 7.0 for 16 hr. at 4°C. The suspension is filtered. The insoluble material is collected and used to fabricate the matrix. The material is mostly collagenous in nature. It is devoid of osteogenic or chondrogenic activity.

3. Matrix Treatments

The major component of all bone matrices is Type-I collagen. In addition to collagen, demineralized bone extracted as disclosed above includes non-collagenous proteins which may account for 5% of its mass. In a xenogenic matrix, these noncollagenous components may present themselves as potent antigens, and may constitute immunogenic and/or inhibitory components. These components also may inhibit osteogenesis in allogenic implants by interfering with the developmental cascade of bone differentiation. It has been discovered that treatment of the matrix particles with a collagen fibril-modifying agent extracts potentially unwanted components from the matrix, and alters the surface structure of the matrix material. Useful agents include acids, organic solvents or heated aqueous media. Various treatments are described below. A detailed physical analysis of the effect these fibril-modifying agents have on demineralized, quanidine-extracted bone collagen particles is disclosed in PCT WO 90/10018, published 7-SEP-90.

After contact with the fibril-modifying agent, the treated matrix is washed to remove any extracted components, following a form of the procedure set forth below:

1. Suspend in TBS (Tris-buffered saline) 1g/200 ml and stir at 4°C for 2 hrs; or in 6 M urea, 50 mM Tris-HCl, 500 mM NaCl, pH 7.0 (UTBS) or water and stir at room temperature (RT) for 30 minutes (sufficient time to neutralize the pH);

2. Centrifuge and repeat wash step; and

3. Centrifuge; discard supernatant; water wash residue; and then lyophilize.

3.1 Acid Treatments

1. Trifluoroacetic acid.

Trifluoroacetic acid is a strong non-oxidizing acid that is a known swelling agent for proteins, and which modifies collagen fibrils.

Bovine bone residue prepared as described above is sieved, and particles of the appropriate size are collected. These particles are extracted with various percentages (1.0% to 100%) of trifluoroacetic acid and water (v/v) at 0°C or room temperature for 1-2 hours with constant stirring. The treated matrix is filtered, lyophilized, or washed with water/salt and then lyophilized.

2. Hydrogen Fluoride.

Like trifluoroacetic acid, hydrogen fluoride is a strong acid and swelling agent, and also is capable of altering intraparticle surface structure. Hydrogen fluoride is also a known deglycosylating agent. As such, HF may function to increase the osteogenic activity of these matrices by removing the antigenic carbohydrate content of any glycoproteins still associated with the matrix after guanidine extraction.

Bovine bone residue prepared as described above is sieved, and particles of the appropriate size are collected. The sample is dried in vacuo over P_2O_5, transferred to the reaction vessel and exposed to anhydrous hydrogen fluoride (10-20 ml/g of matrix) by distillation onto the sample at -70°C. The vessel is allowed to warm to 0°C and the reaction mixture is stirred at this temperature for 120 minutes. After evaporation of the hydrogen fluoride in vacuo, the residue is dried thoroughly in vacuo over KOH pellets to remove any remaining traces of acid. Extent of deglycosylation can be determined from carbohydrate analysis of matrix samples taken before and after treatment with hydrogen fluoride, after washing the samples appropriately to remove non-covalently bound carbohydrates. SDS-extracted protein from HF-treated material is negative for carbohydrate as determined by Con A blotting.

The deglycosylated bone matrix is next washed twice in TBS (Tris-buffered saline) or UTBS, water-washed, and then lyophilized.

Other acid treatments are envisioned in addition to HF and TFA. TFA is a currently preferred acidifying reagent in these treatments because of its volatility. However, it is understood that other, potentially less caustic acids may be used, such as acetic or formic acid.

3.2 Solvent Treatment

1. Dichloromethane.

Dichloromethane (DCM) is an organic solvent capable of denaturing proteins without affecting their primary structure. This swelling agent is a common reagent in automated peptide synthesis, and is used in washing steps to remove components.

Bovine bone residue, prepared as described above, is sieved, and particles of the appropriate size are incubated in 100% DCM or, preferably, 99.9% DCM/0.1% TFA. The matrix is incubated with the swelling agent for one or two hours at 0°C or at room temperature. Alternatively, the matrix is treated with the agent at least three times with short washes (20 minutes each) with no incubation.

2. Acetonitrile.

Acetonitrile (ACN) is an organic solvent, capable of denaturing proteins without affecting their primary structure. It is a common reagent used in high-performance liquid chromatography, and is used to elute proteins from silica-based columns by perturbing hydrophobic interactions.

Bovine bone residue particles of the appropriate size, prepared as described above, are treated with 100% ACN (1.0 g/30 ml) or, preferably, 99.9% ACN/0.1% TFA at room temperature for 1-2 hours with constant stirring. The treated matrix is then water-washed, or washed with urea buffer, or 4 M NaCl and lyophilized. Alternatively, the ACN or ACN/TFA treated matrix may be lyophilized without wash.

3. Isopropanol.

Isopropanol is also an organic solvent capable of denaturing proteins without affecting their primary structure. It is a common reagent used to elute proteins from silica HPLC columns.

Bovine bone residue particles of the appropriate size prepared as described above are treated with 100% isopropanol (1.0 g/30 ml) or, preferably, in the presence of 0.1% TFA, at room temperature for 1-2 hours with constant stirring. The matrix is then water-washed or washed with urea buffer or 4 M NaCl before being lyophilized.

4. Chloroform

Chloroform also may be used to increase surface area of bone matrix like the reagents set forth above, either alone or acidified.

Treatment as set forth above is effective to assure that the material is free of pathogens prior to implantation.

3.3 <u>Heat Treatment</u>

The currently most preferred agent is a heated aqueous fibril-modifying medium such as water, to increase the matrix particle surface area and porosity. The currently most preferred aqueous medium is an acidic aqueous medium having a pH of less than about 4.5, e.g., within the range of pH 2 - pH 4. which may help to "swell" the collagen before heating. 0.1% acetic acid, which has a pH of about 3, currently is preferred. 0.1 M acetic acid also may be used.

Various amounts of delipidated, demineralized guanidine-extracted bone collagen are heated in the aqueous medium (1g matrix/30ml aqueous medium) under constant stirring in a water jacketed glass flask, and maintained at a given temperature for a predetermined period of time. Preferred treatment times are about one hour, although exposure times of between about 0.5 to two hours appear acceptable. The temperature employed is held constant at a temperature generally within the range of about 37°C to 75°C. The currently preferred heat treatment temperature is within the range of 45°C to 60°C.

After the heat treatment, the matrix is filtered, washed, lyophilized and used for implant. Where an acidic aqueous medium is used, the matrix also is preferably neutralized prior to washing and lyophilization. A currently preferred neutralization buffer is a 200mM sodium phosphate buffer, pH 7.0. To neutralize the matrix, the matrix preferably first is allowed to cool following thermal treatment, the acidic aqueous medium (e.g., 0.1% acetic acid) then is removed and replaced with the neutralization buffer and the matrix agitated for about 30 minutes. The neutralization buffer then may be removed and the matrix washed and lyophilized (see infra).

The matrix also may be treated to remove contaminating heavy metals, such as by exposing the matrix to a metal ion chelator. For example, following thermal treatment with 0.1% acetic acid, the matrix may be neutralized in a neutralization buffer containing EDTA (sodium ethylenediaminetetraacetic acid), e.g., 200 mM sodium phosphate, 5mM EDTA, pH 7.0. 5 mM EDTA provides about a 100-fold molar excess of chelator to

residual heavy metals present in the most contaminated matrix tested to date. Subsequent washing of the matrix following neutralization appears to remove the bulk of the EDTA. EDTA treatment of matrix particles reduces the residual heavy metal content of all metals tested (Sb, As, Be, Cd, Cr, Cu, Co, Pb, Hg, Ni, Se, Ag, Zn, Tl) to less than about 1 ppm. Bioassays with EDTA-treated matrices indicate that treatment with the metal ion chelator does not inhibit bone inducing activity.

The collagen matrix materials preferably take the form of a fine powder, insoluble in water, comprising nonadherent particles. It may be used simply by packing into the volume where new bone growth or sustained release is desired, held in place by surrounding tissue. Alternatively, the powder may be encapsulated in, e.g., a gelatin or polylactic acid coating, which is adsorbed readily by the body. The powder may be shaped to a volume of given dimensions and held in that shape by interadhering the particles using, for example, soluble, species-biocompatible collagen. The material may also be produced in sheet, rod, bead, or other macroscopic shapes.

Demineralized rat bone matrix used as an allogenic matrix in certain of the experiments disclosed herein, is prepared from several of the dehydrated diaphyseal shafts of rat femur and tibia as described herein to produce a bone particle size which passes through a 420 μm sieve. The bone particles are subjected to dissociative extraction with 4 M guanidine-HCl. Such treatment results in a complete loss of the inherent ability of the bone matrix to induce endochondral bone differentiation. The remaining insoluble material is used to fabricate the

matrix. The material is mostly collagenous in nature, and upon implantation, does not induce cartilage and bone. All new preparations are tested for mineral content and osteogenic activity before use. The total loss of biological activity of bone matrix is restored when an active osteoinductive protein fraction or a pure osteoinductive protein preparation is reconstituted with the biologically inactive insoluble collagenous matrix.

FABRICATION OF OSTEOGENIC DEVICE

The naturally sourced and recombinant protein as set forth above, and other constructs, can be combined and dispersed in a suitable matrix preparation using any of the methods described below. In general, 50-100 ng of active protein is combined with the inactive carrier matrix (e.g., 25 mg for rat bioassays). Greater amounts may be used for large implants.

1. Ethanol Precipitation

Matrix is added to osteogenic protein dissolved in guanidine-HCl. Samples are vortexed and incubated at a low temperature (e.g., 4°C). Samples are then further vortexed. Cold absolute ethanol (5 volumes) is added to the mixture which is then stirred and incubated, preferably for 30 minutes at -20°C. After centrifugation (microfuge, high speed) the supernatant is discarded. The reconstituted matrix is washed twice with cold concentrated ethanol in water (85% EtOH) and then lyophilized.

2. Acetonitrile Trifluoroacetic

Acid Lyophilization

In this procedure, osteogenic protein in an acetonitrile trifluroacetic acid (ACN/TFA) solution is added to the carrier material. Samples are vigorously vortexed many times and then lyophilized. This method is currently preferred, and has been tested with osteogenic protein at varying concentrations and different levels of purity.

3. Urea Lyophilization

For those osteogenic proteins that are prepared in urea buffer, the protein is mixed with the matrix material, vortexed many times, and then lyophilized. The lyophilized material may be used "as is" for implants.

4. Buffered Saline Lyophilization

OP1 and OP2 preparations in physiological saline may also be vortexed with the matrix and lyophilized to produce osteogenically active material.

These procedures also can be used to adsorb other active therapeutic drugs, hormones, and various bioactive species to the matrix for sustained release purposes.

BIOASSAY

The functioning of the various proteins and devices of this invention can be evaluated with an *in vivo* bioassay. Studies in rats show the osteogenic effect in an appropriate matrix to be dependent on the

dose of osteogenic protein dispersed in the matrix. No activity is observed if the matrix is implanted alone. In vivo bioassays performed in the rat model also have shown that demineralized, guanidine-extracted xenogenic bone matrix materials of the type described in the literature are ineffective as a carrier, fail to induce bone, and produce an inflammatory and immunological response when implanted unless treated as disclosed above. In certain species (e.g., monkey) allogenic matrix materials also apparently are ineffective as carriers. The following sets forth various procedures for preparing osteogenic devices from the proteins and matrix materials prepared as set forth above, and for evaluating their osteogenic utility.

A. Rat Model

1. Implantation

The bioassay for bone induction as described by Sampath and Reddi ((1983) Proc. Natl. Acad. Sci. USA 80 6591-6595), herein incorporated by reference, may be used to monitor endochondral bone differentiation activity. This assay consists of implanting test samples in subcutaneous sites in recipient rats under ether anesthesia. Male Long-Evans rats, aged 28-32 days, were used. A vertical incision (1 cm) is made under sterile conditions in the skin over the thoracic region, and a pocket is prepared by blunt dissection. Approximately 25 mg of the test sample is implanted deep into the pocket and the incision is closed with a metallic skin clip. The day of implantation is designated as day one of the experiment. Implants were removed on day 12. The heterotropic site allows for the study of bone induction without the possible

ambiguities resulting from the use of orthotropic sites. As disclosed herein, both allogenic (rat bone matrix) and xenogenic (bovine bone matrix) implants were assayed.

2. Cellular Events

Successful implants exhibit a controlled progression through the stages of protein-induced endochondral bone development, including: (1) transient infiltration by polymorphonuclear leukocytes on day one; (2) mesenchymal cell migration and proliferation on days two and three; (3) chondrocyte appearance on days five and six; (4) cartilage matrix formation on day seven; (5) cartilage calcification on day eight; (6) vascular invasion, appearance of osteoblasts, and formation of new bone on days nine and ten; (7) appearance of osteoblastic and bone remodeling and dissolution of the implanted matrix on days twelve to eighteen; and (8) hematopoietic bone marrow differentiation in the ossicle on day twenty-one. The results show that the shape of the new bone conforms to the shape of the implanted matrix.

3. Histological Evaluation

Histological sectioning and staining is preferred to determine the extent of osteogenesis in the implants. Implants are fixed in Bouins Solution, embedded in paraffin, and cut into 6-8 μm sections. Staining with toluidine blue or hemotoxylin/eosin demonstrates clearly the ultimate development of endochondral bone. Twelve day implants are usually sufficient to determine whether the implants contain newly induced bone.

4. Biological Markers

Alkaline phosphatase activity may be used as a marker for osteogenesis. The enzyme activity may be determined spectrophotometrically after homogenization of the implant. The activity peaks at 9-10 days *in vivo* and thereafter slowly declines. Implants showing no bone development by histology have little or no alkaline phosphatase activity under these assay conditions. The assay is useful for quantitation and obtaining an estimate of bone formation quickly after the implants are removed from the rat. Alternatively, the amount of bone formation can be determined by measuring the calcium content of the implant.

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The present embodiments are therefore to be considered in all respects as illustrative and not restrictive, the scope of the invention being indicated by the appended claims rather than by the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced therein.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: OPPERMANN, HERMANN
OZKAYNAK, ENGIN
KUBERASAMPATH, THANGAVEL
RUEGER, DAVID C.

(ii) TITLE OF INVENTION: OSTEOGENIC DEVICES

(iii) NUMBER OF SEQUENCES: 9

(iv) CORRESPONDENCE ADDRESS:
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(B) STREET: 53 STATE STREET
(C) CITY: BOSTON
(D) STATE: MASSACHUSETTS
(E) COUNTRY: U.S.A.
(F) ZIP: 02109

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: PITCHER, EDMUND R.
(B) REGISTRATION NUMBER: 27,829
(C) REFERENCE/DOCKET NUMBER: CRR056PC

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 617/248-7000
(B) TELEFAX: 617/248-7100

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1822 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS
(F) TISSUE TYPE: HIPPOCAMPUS

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 49..1341
(C) IDENTIFICATION METHOD: experimental
(D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"
/product= "hOP1-PP"
/evidence= EXPERIMENTAL
/standard_name= "hOP1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys Gln Pro
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280 285 290

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295 300 305

AAG AAC CAG GAA GCC CTG CGG ATG GCC AAC GTG GCA GAG AAC AGC AGC 1017
Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu Asn Ser Ser
310 315 320

AGC GAC CAG AGG CAG GCC TGT AAG AAG CAC GAG CTG TAT GTC AGC TTC 1065
Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe
325 330 335

```
CGA GAC CTG GGC TGG CAG GAC TGG ATC ATC GCG CCT GAA GGC TAC GCC      1113
Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala
340                 345                 350                 355

GCC TAC TAC TGT GAG GGG GAG TGT GCC TTC CCT CTG AAC TCC TAC ATG      1161
Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met
            360                 365                 370

AAC GCC ACC AAC CAC GCC ATC GTG CAG ACG CTG GTC CAC TTC ATC AAC      1209
Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn
        375                 380                 385

CCG GAA ACG GTG CCC AAG CCC TGC TGT GCG CCC ACG CAG CTC AAT GCC      1257
Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala
        390                 395                 400

ATC TCC GTC CTC TAC TTC GAT GAC AGC TCC AAC GTC ATC CTG AAG AAA      1305
Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys
    405                 410                 415

TAC AGA AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCCTCC           1351
Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
420                 425                 430

GAGAATTCAG ACCCTTTGGG GCCAAGTTTT TCTGGATCCT CCATTGCTCG CCTTGGCCAG    1411

GAACCAGCAG ACCAACTGCC TTTTGTGAGA CCTTCCCCTC CCTATCCCCA ACTTTAAAGG    1471

TGTGAGAGTA TTAGGAAACA TGAGCAGCAT ATGGCTTTTG ATCAGTTTTT CAGTGGCAGC    1531

ATCCAATGAA CAAGATCCTA CAAGCTGTGC AGGCAAAACC TAGCAGGAAA AAAAAACAAC    1591

GCATAAAGAA AAATGGCCGG GCCAGGTCAT TGGCTGGGAA GTCTCAGCCA TGCACGGACT    1651

CGTTTCCAGA GGTAATTATG AGCGCCTACC AGCCAGGCCA CCCAGCCGTG GGAGGAAGGG    1711

GGCGTGGCAA GGGGTGGGCA CATTGGTGTC TGTGCGAAAG GAAAATTGAC CCGGAAGTTC    1771

CTGTAATAAA TGTCACAATA AAACGAATGA ATGAAAAAAA AAAAAAAAAA A             1822
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 431 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

(D) OTHER INFORMATION: /Product="hOP1-PP"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala
  1               5                  10                  15

Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser
             20                  25                  30

Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser
         35                  40                  45

Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu
     50                  55                  60

Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro
 65                  70                  75                  80

Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly
                 85                  90                  95

Gly Pro Gly Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser
            100                 105                 110

Thr Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr
        115                 120                 125

Asp Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys
    130                 135                 140

Glu Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu
145                 150                 155                 160

Ser Lys Ile Pro Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile
                165                 170                 175

Tyr Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile
            180                 185                 190

Ser Val Tyr Gln Val Leu Gln Glu His Leu Gly Arg Glu Ser Asp Leu
        195                 200                 205

Phe Leu Leu Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu
    210                 215                 220

Val Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg
225                 230                 235                 240

His Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser
                245                 250                 255

Ile Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn
```

```
                260                 265                 270

Lys Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe
        275                 280                 285

Arg Ser Ile Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser
    290                 295                 300

Lys Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu
305                 310                 315                 320

Asn Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr
                325                 330                 335

Val Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu
            340                 345                 350

Gly Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn
        355                 360                 365

Ser Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His
    370                 375                 380

Phe Ile Asn Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln
385                 390                 395                 400

Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile
                405                 410                 415

Leu Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
            420                 425                 430
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1929 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 103..1293
(D) OTHER INFORMATION: /function= "osteogenic protein"
/product= "mOP2-PP"
/note= "mOP2 cDNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
GAATTCCGCT GCCAGGCACA GGTGCGCCGT CTGGTCCTCC CCGTCTGGCG TCAGCCGAGC      60
```

```
CCGACCAGCT ACCAGTGGAT GCGCGCCGGC TGAAAGTCCG AG ATG GCT ATG CGT        114
                                                Met Ala Met Arg
                                                 1

CCC GGG CCA CTC TGG CTA TTG GGC CTT GCT CTG TGC GCG CTG GGA GGC       162
Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys Ala Leu Gly Gly
 5                  10                  15                  20

GGC CAC GGT CCC GGT CCC CCG CAC ACC TGT CCC CAG CGT CGC CTG GGA       210
Gly His Gly Pro Gly Pro Pro His Thr Cys Pro Gln Arg Arg Leu Gly
                25                  30                  35

GCG CGC GAC CGG GAC ATG CAG CGT GAA ATC CTG CCG GTG CTC GGG CTA       258
Ala Arg Asp Arg Asp Met Gln Arg Glu Ile Leu Pro Val Leu Gly Leu
            40                  45                  50

CCG GGA CGC CCC GAC CCC GTG CAC AAC CCG CCG CTG CCC GGC ACG CAG       306
Pro Gly Arg Pro Asp Pro Val His Asn Pro Pro Leu Pro Gly Thr Gln
        55                  60                  65

CGT GCG CCC CTC TTC ATG TTG GAC CTA TAC CAC GCC ATG ACC GAT GAC       354
Arg Ala Pro Leu Phe Met Leu Asp Leu Tyr His Ala Met Thr Asp Asp
    70                  75                  80

GAC GAC GGC GGG CCA CCA CAG GCT CAC TTA GGC CGT GCC GAC CTG GTC       402
Asp Asp Gly Gly Pro Pro Gln Ala His Leu Gly Arg Ala Asp Leu Val
85                  90                  95                 100

ATG AGC TTC GTC AAC ATG GTG GAA CGC GAC CGT ACC CTG GGC TAC CAG       450
Met Ser Phe Val Asn Met Val Glu Arg Asp Arg Thr Leu Gly Tyr Gln
                105                 110                 115

GAG CCA CAC TGG AAG GAA TTC CAC TTT GAC CTA ACC CAG ATC CCT GCT       498
Glu Pro His Trp Lys Glu Phe His Phe Asp Leu Thr Gln Ile Pro Ala
            120                 125                 130

GGG GAG GCT GTC ACA GCT GCT GAG TTC CGG ATC TAC AAA GAA CCC AGC       546
Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Glu Pro Ser
        135                 140                 145

ACC CAC CCG CTC AAC ACA ACC CTC CAC ATC AGC ATG TTC GAA GTG GTC       594
Thr His Pro Leu Asn Thr Thr Leu His Ile Ser Met Phe Glu Val Val
    150                 155                 160

CAA GAG CAC TCC AAC AGG GAG TCT GAC TTG TTC TTT TTG GAT CTT CAG       642
Gln Glu His Ser Asn Arg Glu Ser Asp Leu Phe Phe Leu Asp Leu Gln
165                 170                 175                 180

ACG CTC CGA TCT GGG GAC GAG GGC TGG CTG GTG CTG GAC ATC ACA GCA       690
Thr Leu Arg Ser Gly Asp Glu Gly Trp Leu Val Leu Asp Ile Thr Ala
                185                 190                 195

GCC AGT GAC CGA TGG CTG CTG AAC CAT CAC AAG GAC CTG GGA CTC CGC       738
```

```
Ala Ser Asp Arg Trp Leu Leu Asn His His Lys Asp Leu Gly Leu Arg
            200                 205                 210

CTC TAT GTG GAA ACC GCG GAT GGG CAC AGC ATG GAT CCT GGC CTG GCT       786
Leu Tyr Val Glu Thr Ala Asp Gly His Ser Met Asp Pro Gly Leu Ala
        215                 220                 225

GGT CTG CTT GGA CGA CAA GCA CCA CGC TCC AGA CAG CCT TTC ATG GTA       834
Gly Leu Leu Gly Arg Gln Ala Pro Arg Ser Arg Gln Pro Phe Met Val
    230                 235                 240

ACC TTC TTC AGG GCC AGC CAG AGT CCT GTG CGG GCC CCT CGG GCA GCG       882
Thr Phe Phe Arg Ala Ser Gln Ser Pro Val Arg Ala Pro Arg Ala Ala
245                 250                 255                 260

AGA CCA CTG AAG AGG AGG CAG CCA AAG AAA ACG AAC GAG CTT CCG CAC       930
Arg Pro Leu Lys Arg Arg Gln Pro Lys Lys Thr Asn Glu Leu Pro His
            265                 270                 275

CCC AAC AAA CTC CCA GGG ATC TTT GAT GAT GGC CAC GGT TCC CGC GGC       978
Pro Asn Lys Leu Pro Gly Ile Phe Asp Asp Gly His Gly Ser Arg Gly
            280                 285                 290

AGA GAG GTT TGC CGC AGG CAT GAG CTC TAC GTC AGA TTC CGT GAC CTT      1026
Arg Glu Val Cys Arg Arg His Glu Leu Tyr Val Arg Phe Arg Asp Leu
        295                 300                 305

GGC TGG CTG GAC TGG GTC ATC GCC CCC CAG GGC TAC TCT GCC TAT TAC      1074
Gly Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr
    310                 315                 320

TGT GAG GGG GAG TGT GCT TTC CCA CTG GAC TCC TGT ATG AAC GCC ACC      1122
Cys Glu Gly Glu Cys Ala Phe Pro Leu Asp Ser Cys Met Asn Ala Thr
325                 330                 335                 340

AAC CAT GCC ATC TTG CAG TCT CTG GTG CAC CTG ATG AAG CCA GAT GTT      1170
Asn His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro Asp Val
                345                 350                 355

GTC CCC AAG GCA TGC TGT GCA CCC ACC AAA CTG AGT GCC ACC TCT GTG      1218
Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr Ser Val
            360                 365                 370

CTG TAC TAT GAC AGC AGC AAC AAT GTC ATC CTG CGT AAA CAC CGT AAC      1266
Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His Arg Asn
        375                 380                 385

ATG GTG GTC AAG GCC TGT GGC TGC CAC TGAGGCCCCG CCCAGCATCC            1313
Met Val Val Lys Ala Cys Gly Cys His
    390                 395

TGCTTCTACT ACCTTACCAT CTGGCCGGGC CCCTCTCCAG AGGCAGAAAC CCTTCTATGT    1373
```

TATCATAGCT CAGACAGGGG CAATGGGAGG CCCTTCACTT CCCCTGGCCA CTTCCTGCTA 1433

AAATTCTGGT CTTTCCCAGT TCCTCTGTCC TTCATGGGGT TTCGGGGCTA TCACCCCGCC 1493

CTCTCCATCC TCCTACCCCA AGCATAGACT GAATGCACAC AGCATCCCAG AGCTATGCTA 1553

ACTGAGAGGT CTGGGGTCAG CACTGAAGGC CCACATGAGG AAGACTGATC CTTGGCCATC 1613

CTCAGCCCAC AATGGCAAAT TCTGGATGGT CTAAGAAGCC CTGGAATTCT AAACTAGATG 1673

ATCTGGGCTC TCTGCACCAT TCATTGTGGC AGTTGGGACA TTTTTAGGTA TAACAGACAC 1733

ATACACTTAG ATCAATGCAT CGCTGTACTC CTTGAAATCA GAGCTAGCTT GTTAGAAAAA 1793

GAATCAGAGC CAGGTATAGC GGTGCATGTC ATTAATCCCA GCGCTAAAGA GACAGAGACA 1853

GGAGAATCTC TGTGAGTTCA AGGCCACATA GAAAGAGCCT GTCTCGGGAG CAGGAAAAAA 1913

AAAAAAAACG GAATTC 1929

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 397 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(D) OTHER INFORMATION: /Product= "mOP2-PP"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Ala Met Arg Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys
1 5 10 15

Ala Leu Gly Gly Gly His Gly Pro Gly Pro Pro His Thr Cys Pro Gln
20 25 30

Arg Arg Leu Gly Ala Arg Asp Arg Asp Met Gln Arg Glu Ile Leu Pro
35 40 45

Val Leu Gly Leu Pro Gly Arg Pro Asp Pro Val His Asn Pro Pro Leu
50 55 60

Pro Gly Thr Gln Arg Ala Pro Leu Phe Met Leu Asp Leu Tyr His Ala
65 70 75 80

Met Thr Asp Asp Asp Asp Gly Gly Pro Pro Gln Ala His Leu Gly Arg
85 90 95

Ala Asp Leu Val Met Ser Phe Val Asn Met Val Glu Arg Asp Arg Thr
100 105 110

Leu Gly Tyr Gln Glu Pro His Trp Lys Glu Phe His Phe Asp Leu Thr
115 120 125

Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr
130 135 140

Lys Glu Pro Ser Thr His Pro Leu Asn Thr Thr Leu His Ile Ser Met
145 150 155 160

Phe Glu Val Val Gln Glu His Ser Asn Arg Glu Ser Asp Leu Phe Phe
165 170 175

Leu Asp Leu Gln Thr Leu Arg Ser Gly Asp Glu Gly Trp Leu Val Leu
180 185 190

Asp Ile Thr Ala Ala Ser Asp Arg Trp Leu Leu Asn His His Lys Asp
195 200 205

Leu Gly Leu Arg Leu Tyr Val Glu Thr Ala Asp Gly His Ser Met Asp
210 215 220

Pro Gly Leu Ala Gly Leu Leu Gly Arg Gln Ala Pro Arg Ser Arg Gln
225 230 235 240

Pro Phe Met Val Thr Phe Phe Arg Ala Ser Gln Ser Pro Val Arg Ala
245 250 255

Pro Arg Ala Ala Arg Pro Leu Lys Arg Arg Gln Pro Lys Lys Thr Asn
260 265 270

Glu Leu Pro His Pro Asn Lys Leu Pro Gly Ile Phe Asp Asp Gly His
275 280 285

Gly Ser Arg Gly Arg Glu Val Cys Arg Arg His Glu Leu Tyr Val Arg
290 295 300

Phe Arg Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr
305 310 315 320

Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asp Ser Cys
325 330 335

Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His Leu Met
340 345 350

Lys Pro Asp Val Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser
355 360 365

Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg
370 375 380

```
Lys His Arg Asn Met Val Val Lys Ala Cys Gly Cys His
385                 390                 395
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1941 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS
(F) TISSUE TYPE: HIPPOCAMPUS

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 507..1703
(D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"
/product= "hOP2-PP"
/note= "hOP2 (CDNA)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
GGAATTCCGG CCACAGTGGC GCCGGCAGAG CAGGAGTGGC TGGAGGAGCT GTGGTTGGAG       60

CAGGAGGTGG CACGGCAGGG CTGGAGGGCT CCCTATGAGT GGCGGAGACG GCCCAGGAGG      120

CGCTGGAGCA ACAGCTCCCA CACCGCACCA AGCGGTGGCT GCAGGAGCTC GCCCATCGCC      180

CCTGCGCTGC TCGGACCGCG GCCACAGCCG GACTGGCGGG TACGGCGGCG ACAGAGGCAT      240

TGGCCGAGAG TCCCAGTCCG CAGAGTAGCC CCGGCCTCGA GGCGGTGGCG TCCCGGTCCT      300

CTCCGTCCAG GAGCCAGGAC AGGTGTCGCG CGGCGGGGCT CCAGGGACCG CGCCTGAGGC      360

CGGCTGCCCG CCCGTCCCGC CCCGCCCCGC CGCCCGCCGC CCGCCGAGCC CAGCCTCCTT      420

GCCGTCGGGG CGTCCCCAGG CCCTGGGTCG GCCGCGGAGC CGATGCGCGC CCGCTGAGCG      480

CCCCAGCTGA GCGCCCCCGG CCTGCC ATG ACC GCG CTC CCC GGC CCG CTC TGG      533
                             Met Thr Ala Leu Pro Gly Pro Leu Trp
                              1               5

CTC CTG GGC CTG GCG CTA TGC GCG CTG GGC GGG GGC GGC CCC GGC CTG      581
Leu Leu Gly Leu Ala Leu Cys Ala Leu Gly Gly Gly Gly Pro Gly Leu
 10                  15                  20                  25

CGA CCC CCG CCC GGC TGT CCC CAG CGA CGT CTG GGC GCG CGC GAC CGG      629
```

Arg Pro Pro Pro Gly Cys Pro Gln Arg Arg Leu Gly Ala Arg Asp Arg
30 35 40

GAC GTG CAG CGC GAG ATC CTG GCG GTG CTC GGG CTG CCT GGG CGG CCC 677
Asp Val Gln Arg Glu Ile Leu Ala Val Leu Gly Leu Pro Gly Arg Pro
45 50 55

CGG CCC CGC GCG CCA CCC GCC GCC TCC CGG CTG CCC GCG TCC GCG CCG 725
Arg Pro Arg Ala Pro Pro Ala Ala Ser Arg Leu Pro Ala Ser Ala Pro
60 65 70

CTC TTC ATG CTG GAC CTG TAC CAC CGC ATG GCC GGC GAC GAC GAC GAG 773
Leu Phe Met Leu Asp Leu Tyr His Arg Met Ala Gly Asp Asp Asp Glu
75 80 85

GAC GGC GCC GCG GAG GCC CTG GGC CGC GCC GAC CTG GTC ATG AGC TTC 821
Asp Gly Ala Ala Glu Ala Leu Gly Arg Ala Asp Leu Val Met Ser Phe
90 95 100 105

GTT AAC ATG GTG GAG CGA GAC CGT GCC CTG GGC CAC CAG GAG CCC CAT 869
Val Asn Met Val Glu Arg Asp Arg Ala Leu Gly His Gln Glu Pro His
110 115 120

TGG AAG GAG TTC CGC TTT GAC CTG ACC CAG ATC CCG GCT GGG GAG GCG 917
Trp Lys Glu Phe Arg Phe Asp Leu Thr Gln Ile Pro Ala Gly Glu Ala
125 130 135

GTC ACA GCT GCG GAG TTC CGG ATT TAC AAG GTG CCC AGC ATC CAC CTG 965
Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Val Pro Ser Ile His Leu
140 145 150

CTC AAC AGG ACC CTC CAC GTC AGC ATG TTC CAG GTG GTC CAG GAG CAG 1013
Leu Asn Arg Thr Leu His Val Ser Met Phe Gln Val Val Gln Glu Gln
155 160 165

TCC AAC AGG GAG TCT GAC TTG TTC TTT TTG GAT CTT CAG ACG CTC CGA 1061
Ser Asn Arg Glu Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr Leu Arg
170 175 180 185

GCT GGA GAC GAG GGC TGG CTG GTG CTG GAT GTC ACA GCA GCC AGT GAC 1109
Ala Gly Asp Glu Gly Trp Leu Val Leu Asp Val Thr Ala Ala Ser Asp
190 195 200

TGC TGG TTG CTG AAG CGT CAC AAG GAC CTG GGA CTC CGC CTC TAT GTG 1157
Cys Trp Leu Leu Lys Arg His Lys Asp Leu Gly Leu Arg Leu Tyr Val
205 210 215

GAG ACT GAG GAC GGG CAC AGC GTG GAT CCT GGC CTG GCC GGC CTG CTG 1205
Glu Thr Glu Asp Gly His Ser Val Asp Pro Gly Leu Ala Gly Leu Leu
220 225 230

GGT CAA CGG GCC CCA CGC TCC CAA CAG CCT TTC GTG GTC ACT TTC TTC 1253
Gly Gln Arg Ala Pro Arg Ser Gln Gln Pro Phe Val Val Thr Phe Phe

```
     235                 240                 245

AGG GCC AGT CCG AGT CCC ATC CGC ACC CCT CGG GCA GTG AGG CCA CTG      1301
Arg Ala Ser Pro Ser Pro Ile Arg Thr Pro Arg Ala Val Arg Pro Leu
250                 255                 260                 265

AGG AGG AGG CAG CCG AAG AAA AGC AAC GAG CTG CCG CAG GCC AAC CGA      1349
Arg Arg Arg Gln Pro Lys Lys Ser Asn Glu Leu Pro Gln Ala Asn Arg
                270                 275                 280

CTC CCA GGG ATC TTT GAT GAC GTC CAC GGC TCC CAC GGC CGG CAG GTC      1397
Leu Pro Gly Ile Phe Asp Asp Val His Gly Ser His Gly Arg Gln Val
            285                 290                 295

TGC CGT CGG CAC GAG CTC TAC GTC AGC TTC CAG GAC CTC GGC TGG CTG      1445
Cys Arg Arg His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu
        300                 305                 310

GAC TGG GTC ATC GCT CCC CAA GGC TAC TCG GCC TAT TAC TGT GAG GGG      1493
Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly
    315                 320                 325

GAG TGC TCC TTC CCA CTG GAC TCC TGC ATG AAT GCC ACC AAC CAC GCC      1541
Glu Cys Ser Phe Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala
330                 335                 340                 345

ATC CTG CAG TCC CTG GTG CAC CTG ATG AAG CCA AAC GCA GTC CCC AAG      1589
Ile Leu Gln Ser Leu Val His Leu Met Lys Pro Asn Ala Val Pro Lys
                350                 355                 360

GCG TGC TGT GCA CCC ACC AAG CTG AGC GCC ACC TCT GTG CTC TAC TAT      1637
Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr
            365                 370                 375

GAC AGC AGC AAC AAC GTC ATC CTG CGC AAA GCC CGC AAC ATG GTG GTC      1685
Asp Ser Ser Asn Asn Val Ile Leu Arg Lys Ala Arg Asn Met Val Val
        380                 385                 390

AAG GCC TGC GGC TGC CAC TGAGTCAGCC CGCCCAGCCC TACTGCAGCA             1733
Lys Ala Cys Gly Cys His
    395

ATTCACTGGC CGTCGTTTTA CAACGTGTGA CTGGGAAAAC CCTGGCGTTA CCCAACTTAA    1793

TCGCCTTGCA GCACATCCCC CTTTCGCCAG CTGGCTAATA GCGAAGAGGC CCCGCACCGA    1853

TCGCCCTTCC CAACAGTTGC GCCCCAGTGA ATGGCGAATG GCAAATTGTA AGCGTTAATA    1913

TTTTGTTAAA ATTCGCGTTA AATTTTTT                                       1941
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 399 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(D) OTHER INFORMATION: /product= "hOP2-PP"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Thr Ala Leu Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys
 1               5                  10                  15

Ala Leu Gly Gly Gly Gly Pro Gly Leu Arg Pro Pro Pro Gly Cys Pro
            20                  25                  30

Gln Arg Arg Leu Gly Ala Arg Asp Arg Asp Val Gln Arg Glu Ile Leu
        35                  40                  45

Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg Ala Pro Pro Ala
    50                  55                  60

Ala Ser Arg Leu Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu Tyr
65                  70                  75                  80

His Arg Met Ala Gly Asp Asp Asp Glu Asp Gly Ala Ala Glu Ala Leu
                85                  90                  95

Gly Arg Ala Asp Leu Val Met Ser Phe Val Asn Met Val Glu Arg Asp
            100                 105                 110

Arg Ala Leu Gly His Gln Glu Pro His Trp Lys Glu Phe Arg Phe Asp
        115                 120                 125

Leu Thr Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala Glu Phe Arg
    130                 135                 140

Ile Tyr Lys Val Pro Ser Ile His Leu Leu Asn Arg Thr Leu His Val
145                 150                 155                 160

Ser Met Phe Gln Val Val Gln Glu Gln Ser Asn Arg Glu Ser Asp Leu
                165                 170                 175

Phe Phe Leu Asp Leu Gln Thr Leu Arg Ala Gly Asp Glu Gly Trp Leu
            180                 185                 190

Val Leu Asp Val Thr Ala Ala Ser Asp Cys Trp Leu Leu Lys Arg His
        195                 200                 205

Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu Thr Glu Asp Gly His Ser
    210                 215                 220
```

```
Val Asp Pro Gly Leu Ala Gly Leu Leu Gly Gln Arg Ala Pro Arg Ser
225                 230                 235                 240

Gln Gln Pro Phe Val Val Thr Phe Phe Arg Ala Ser Pro Ser Pro Ile
                    245                 250                 255

Arg Thr Pro Arg Ala Val Arg Pro Leu Arg Arg Arg Gln Pro Lys Lys
            260                 265                 270

Ser Asn Glu Leu Pro Gln Ala Asn Arg Leu Pro Gly Ile Phe Asp Asp
        275                 280                 285

Val His Gly Ser His Gly Arg Gln Val Cys Arg Arg His Glu Leu Tyr
    290                 295                 300

Val Ser Phe Gln Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln
305                 310                 315                 320

Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ser Phe Pro Leu Asp
                325                 330                 335

Ser Cys Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His
            340                 345                 350

Leu Met Lys Pro Asn Ala Val Pro Lys Ala Cys Cys Ala Pro Thr Lys
        355                 360                 365

Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile
    370                 375                 380

Leu Arg Lys Ala Arg Asn Met Val Val Lys Ala Cys Gly Cys His
385                 390                 395
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 102 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..102
(D) OTHER INFORMATION: /label= OPX
/note= "WHEREIN EACH XAA IS INDEPENDENTLY SELECTED FROM A GROUP OF ONE OR MORE SPECIFIED AMINO ACIDS AS DEFINED IN THE SPECIFICATION

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Cys Xaa Xaa His Glu Leu Tyr Val Xaa Phe Xaa Asp Leu Gly Trp Xaa
 1               5                  10                  15

Asp Trp Xaa Ile Ala Pro Xaa Gly Tyr Xaa Ala Tyr Tyr Cys Glu Gly
            20                  25                  30

Glu Cys Xaa Phe Pro Leu Xaa Ser Xaa Met Asn Ala Thr Asn His Ala
        35                  40                  45

Ile Xaa Gln Xaa Leu Val His Xaa Xaa Xaa Pro Xaa Xaa Val Pro Lys
    50                  55                  60

Xaa Cys Cys Ala Pro Thr Xaa Leu Xaa Ala Xaa Ser Val Leu Tyr Xaa
65                  70                  75                  80

Asp Xaa Ser Xaa Asn Val Xaa Leu Xaa Lys Xaa Arg Asn Met Val Val
                85                  90                  95

Xaa Ala Cys Gly Cys His
            100
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 97 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..97
(D) OTHER INFORMATION: /label= OPX-7C
/note= "WHEREIN EACH XAA INDEPENDENTLY INDICATES ONE OF THE 20 NATURALLY-OCCURRING L-ISOMER, A-AMINO ACIDS, OR A DERIVATIVE THEREOF."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
 1               5                  10                  15

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Cys Xaa Xaa Xaa
            20                  25                  30

Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
        35                  40                  45

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Cys Xaa Xaa
    50                  55                  60
```

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
65 70 75 80

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Cys
85 90 95

Xaa

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 102 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: Protein
(B) LOCATION: 1..102
(D) OTHER INFORMATION: /label= PROTEIN
/note= "WHEREIN EACH XAA INDEPENDENTLY INDICATES ONE OF THE 20 NATURALLY-OCCURRING L-ISOMER A-AMINO AICDS, OR A DERIVATIVE THEREOF."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5 10 15

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa
20 25 30

Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa
35 40 45

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
50 55 60

Xaa Cys Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
65 70 75 80

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
85 90 95

Xaa Xaa Cys Xaa Cys Xaa
100

What is claimed is:

1. A polypeptide chain comprising an amino acid sequence described by residues 303-399 of Seq. ID No. 5.

2. The polypeptide chain of claim 1 comprising an amino acid sequence described by residues 297-399 of Seq. ID No. 5.

3. The polypeptide chain of claim 2 comprising of amino acid sequence described by residues 267-399 of Seq. ID No. 5.

4. The polypeptide chain of claim 3 comprising an amino acid sequence described by residues 264-399 of Seq. ID No. 5.

5. The polypeptide chain of claim 4 comprising an amino acid sequence described by residues 240-399 of Seq. ID No. 5.

6. The polypeptide chain of claim 5 comprising an amino acid sequence described by residues 1-399 of Seq. ID No. 5.

7. A polypeptide chain comprising an amino acid sequence described by residues of 301-397 of Seq. ID No. 3.

8. The polypeptide chain of claim 7 comprising an amino acid sequence described by residues 296-397 of Seq. ID No.3.

9. The polypeptide chain of claim 8 comprising an amino acid sequence described by residues 259-397 of Seq. ID No. 3.

10. The polypeptide chain of claim 9 comprising an amino acid described by residues 1-397 of Seq. ID No. 3.

11. A polypeptide chain useful as a subunit of a dimeric osteogenic protein comprising a pair of disulfide-bonded polypeptide chains, said polypeptide chain having an amino acid sequence described by residues 303-399 of Seq. ID No. 5, including allelic and species variants thereof, such that the dimeric osteogenic protein comprising said polypeptide chain has a conformation capable of inducing endochondral bone formation when implanted in a mammal in association with a matrix.

12. The polypeptide chain of claim 11 wherein said amino acid sequence comprises residues 261-399 of Seq. ID 5.

13. The polypeptide chain of claim 11 wherein the amino acid sequence comprises residues 301-397 of Seq. ID No. 3.

14. The polypeptide chain of claim 13 wherein said amino acid sequent comprises residues 259-397 of Seq. ID No. 3.

15. A dimeric osteogenic protein capable of inducing endochondral bone formation in a mammal when implanted in said mammal in association with a matrix;

said protein comprising a pair of disulfide-bonded polypeptide chains constituting a dimeric species, wherein each said polypeptide chain is the polypeptide chain of claim 11.

16. The polypeptide chain of claim 3 or 11 produced by expression of recombinant DNA in a host cell.

17. The polypeptide chain of claim 16 wherein said host cell is a procaryotic host cell.

18. The polypeptide chain of claim 16 wherein said host cell is a mammalian cell.

19. The polypeptide of claim 1, 3 or 11 that is glycosylated.

20. A nucleic acid encoding the polypeptide chain of claim 1, 3, or 11.

21. A dimeric protein comprising a pair of polypeptide chains expressed from a DNA sequence described by ID No. 3 or ID No. 5, including allelic and species variants thereof, such that, when said polypeptide chains are oxidized to produce a disulfide-bonded dimeric species, the dimeric species has a conformation that is capable of inducing endochondral bone or cartilage formation when disposed within a matrix and implanted in a mammal.

hOP2	Ala	Val	Arg	Pro	Leu	Arg	Arg	Arg
mOP2	...	Ala	...	...	...	Lys	...	...
					5			

hOP2	Gln	Pro	Lys	Lys	Ser	Asn	Glu	Leu
mOP2	...	...	...	...	Thr	...	...	...
		10					15	

hOP2	Pro	Gln	Ala	Asn	Arg	Leu	Pro	Gly
mOP2	...	His	Pro	...	Lys	...	...	...
				20				

hOP2	Ile	Phe	Asp	Asp	Val	His	Gly	Ser
mOP2	...	...	...	...	Gly	...	...	...
	25					30		

hOP2	His	Gly	Arg	Gln	Val	Cys	Arg	Arg
mOP2	Arg	...	...	Glu	...	...	...	...
			35					40

hOP2	His	Glu	Leu	Tyr	Val	Ser	Phe	Gln
mOP2	...	...	...	...	...	Arg	...	Arg
					45			

hOP2	Asp	Leu	Gly	Trp	Leu	Asp	Trp	Val
mOP2	...	...	...	...	...	...	...	...
		50					55	

hOP2	Ile	Ala	Pro	Gln	Gly	Tyr	Ser	Ala
mOP2	...	...	...	...	...	...	...	...
				60				

hOP2	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ser
mOP2	...	...	...	...	...	...	...	Ala
	65					70		

Fig. 1.1

```
hOP2   Phe Pro Leu Asp Ser Cys Met Asn
mOP2   ... ... ... ... ... ... ... ...
                75                  80

hOP2   Ala Thr Asn His Ala Ile Leu Gln
mOP2   ... ... ... ... ... ... ... ...
                        85

hOP2   Ser Leu Val His Leu Met Lys Pro
mOP2   ... ... ... ... ... ... ... ...
           90                  95

hOP2   Asn Ala Val Pro Lys Ala Cys Cys
mOP2   Asp Val ... ... ... ... ... ...
               100

hOP2   Ala Pro Thr Lys Leu Ser Ala Thr
mOP2   ... ... ... ... ... ... ... ...
       105                 110

hOP2   Ser Val Leu Tyr Tyr Asp Ser Ser
mOP2   ... ... ... ... ... ... ... ...
               115                 120

hOP2   Asn Asn Val Ile Leu Arg Lys Ala
mOP2   ... ... ... ... ... ... ... His
                       125

hOP2   Arg Asn Met Val Val Lys Ala Cys
mOP2   ... ... ... ... ... ... ... ...
           130                 135

hOP2   Gly Cys His
mOP2   ... ... ...
```

Fig. 1.2

hOP1	Ser	Thr	Gly	Ser	Lys	Gln	Arg	Ser	Gln
mOP1	...	...	...	Gly	...	...	...	...	...
hOP2	Ala	Val	Arg	Pro	Leu	Arg	...	Arg	...
mOP2	Ala	Ala	Arg	Pro	Leu	Lys	...	Arg	...
	1				5				

hOP1	Asn	Arg	Ser	Lys	Thr	Pro	Lys	Asn	Gln
mOP1	...	...	...	...	...	...	...	...	...
hOP2	Pro	Lys	Lys	Ser	Asn	Glu	Leu	Pro	Gln
mOP2	Pro	Lys	Lys	Thr	Asn	Glu	Leu	Pro	His
	10					15			

hOP1	Glu	Ala	Leu	Arg	Met	Ala	Asn	Val	Ala
mOP1	...	...	...	...	...	...	Ser	...	...
hOP2	Ala	Asn	Arg	Leu	Pro	Gly	Ile	Phe	Asp
mOP2	Pro	Asn	Lys	Leu	Pro	Gly	Ile	Phe	Asp
		20					25		

hOP1	Glu	Asn	Ser	Ser	Ser	Asp	Gln	Arg	Gln
mOP1	...	...	...	...	...	...	...	...	...
hOP2	Asp	Val	His	Gly	...	His	Gly	...	...
mOP2	Asp	Gly	His	Gly	...	Arg	Gly	...	Glu
			30					35	

hOP1	Ala	Cys	Lys	Lys	His	Glu	Leu	Tyr	Val
mOP1	...	...	...	...	...	...	...	...	...
hOP2	Val	...	Arg	Arg	...	...	...	...	...
mOP2	Val	...	Arg	Arg	...	...	...	...	...
				40					45

Fig. 2.1

hOP1	Ser	Phe	Arg	Asp	Leu	Gly	Trp	Gln	Asp
mOP1	...	...	...	...	...	...	...	...	...
hOP2	...	...	Gln	...	...	...	...	Leu	...
mOP2	Arg	...	...	...	...	...	...	Leu	...
					10				

hOP1	Trp	Ile	Ile	Ala	Pro	Glu	Gly	Tyr	Ala
mOP1	...	...	...	...	...	...	...	...	...
hOP2	...	Val	...	...	...	Gln	...	...	Ser
mOP2	...	Val	...	...	...	Gln	...	...	Ser
	55					60			

hOP1	Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala
mOP1	...	...	...	...	...	...	...	...	...
hOP2	...	...	...	...	...	...	...	...	Ser
mOP2	...	...	...	...	...	...	...	...	...
		65					70		

hOP1	Phe	Pro	Leu	Asn	Ser	Tyr	Met	Asn	Ala
mOP1	...	...	...	...	...	...	...	...	...
hOP2	...	...	...	Asp	...	Cys	...	...	...
mOP2	...	...	...	Asp	...	Cys	...	...	...
			75					80	

hOP1	Thr	Asn	His	Ala	Ile	Val	Gln	Thr	Leu
mOP1	...	...	...	...	...	...	...	...	...
hOP2	...	...	...	...	...	Leu	...	Ser	...
mOP2	...	...	...	...	...	Leu	...	Ser	...
				85					90

Fig. 2.2

hOP1	Val	His	Phe	Ile	Asn	Pro	Glu	Thr	Val
mOP1	...	...	...	...	...	...	Asp	...	...
hOP2	...	...	Leu	Met	Lys	...	Asn	Ala	...
mOP2	...	...	Leu	Met	Lys	...	Asp	Val	...
					95				

hOP1	Pro	Lys	Pro	Cys	Cys	Ala	Pro	Thr	Gln
mOP1	...	...	...	...	...	...	...	...	...
hOP2	...	...	Ala	...	...	...	...	...	Lys
mOP2	...	...	Ala	...	...	...	...	...	Lys
	100					105			

hOP1	Leu	Asn	Ala	Ile	Ser	Val	Leu	Tyr	Phe
mOP1	...	...	...	...	...	...	...	...	...
hOP2	...	Ser	...	Thr	...	...	...	...	Tyr
mOP2	...	Ser	...	Thr	...	...	...	...	Tyr
		110					115		

hOP1	Asp	Asp	Ser	Ser	Asn	Val	Ile	Leu	Lys
mOP1	...	...	...	...	...	...	Asp	...	...
hOP2	...	Ser	...	Asn	...	...	...	...	Arg
mOP2	...	Ser	...	Asn	...	...	...	...	Arg
			120					125	

hOP1	Lys	Tyr	Arg	Asn	Met	Val	Val	Arg
mOP1	...	...	...	...	...	...	...	...
hOP2	...	Ala	...	...	...	...	...	Lys
mOP2	...	His	...	...	...	...	...	Lys
				130				

hOP1	Ala	Cys	Gly	Cys	His
mOP1	...	...	...	...	...
hOP2	...	...	...	...	...
mOP2	...	...	...	...	...
	135				

Fig. 2.3

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 91/07635

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.5 C 12 N 15/00 C 07 K 7/10 C 07 K 13/00
A 61 K 37/02 A 61 K 27/00

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl.5	C 07 K A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X A	WO,A,8909788 (CREATIVE BIOMOLECULES) 19 October 1989, see the whole dcument ---	11,15-21 1-10,12-14
X A	WO,A,9011366 (GENETICS INSTITUTE) 4 October 1990, see the whole document ---	11,15-21 11,15-21
X	EMBO Journal, volume 9, no. 7, 1990, Oxford University Press (Eynsham, Oxford, GB) E. Ozkaynak et al.: "OP-1 cDNA encodes an osteogenic protein in the TGF-beta familiy", pages 2085-2093, see the whole article -----	11,15-21

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
31-01-1992	20 FEB 1992
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	MISS T. TAZELAAR

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 9107635 SA 53017

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 12/02/92
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		US-A- 5011691	30-04-91
		AU-A- 3444989	03-11-89
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		US-A- 4975526	04-12-90
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		CA-A- 2030518	29-09-90
		EP-A- 0429570	05-06-91

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For more details about this annex : see Official Journal of the European Patent Office, No. 12/82